SPECIAL ARTICLES

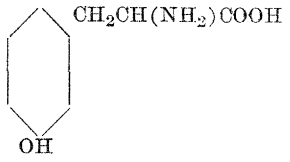
IODINE AND ANURAN METAMORPHOSIS

THE following experiment shows clearly the importance of iodine in inducing anuran metamorphosis and indicates that other halogens such as bromine can not be substituted for it.

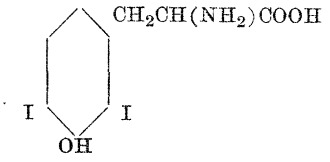
Thyroidectomized and hypophysectomized *R. sylvatica,* the glands of which had been extirpated early in embryonic life, were kept until after the normal time for metamorphosis had elapsed and the normal controls had transformed, and then divided into three sets for experimental purposes. It will be recalled that thyroidless and pituitaryless larvæ do not metamorphose but remain permanent tadpoles unless fed thyroid derivatives or very large amounts of iodine. One set of animals was fed quantities of pure tyrosine $CH_2CH(NH_2)COOH$ each day and allowed to remain in weak solutions of this substance six to eight hours every day. The remainder of the time they were kept in large glass containers and fed large quantities of spirogyra.

The second lot of animals was fed equal quantities of tyrosine in which two atoms of iodine had been substituted for two hydrogen atoms of the tyrosine—forming the well-known compound, 3-5-di-iodo-tyrosine. The iodine in this compound is, of course, an integral part of the molecule.

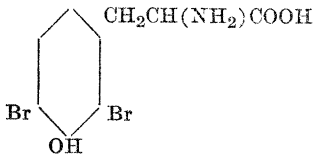
Tyrosine



3-5-diiodotyrosine



The third set of tadpoles was fed quantities of tyrosine equal in amount to that received by the other two cultures, in which two atoms of bromine had been substituted for two hydrogen atoms of the tyrosine molecule forming the compound 3-5-di-brom-tyrosine.



The di-brom-tyrosine was prepared according to the method of C. T. Mörner, 1913, *Zeitschrift für physiologische Chemie,* Vol. 88.

The animals fed tyrosine and spirogyra showed no changes indicative of metamorphosis after sixty days of continuous feeding, though the normal growth rate was not interrupted. After the twenty-fifth day the hind legs differentiated and grew very slowly until they attained a length of 3 to 5 millimeters. There were no signs of fore legs, skin autolysis in the pectoral region, tail shrinkage or anything suggesting transformation.

The animals fed 3-5-di-brom-tyrosine behaved in identical fashion with those of the tyrosine-fed culture and no metamorphosis resulted. At the end of the second month of feeding the hind legs of the animals of the culture averaged five millimeters. The growth rate of the animals was not interfered with by the brom-tyrosine compound. Insofar as the acceleration of metamorphosis is concerned, the results obtained by feeding tyrosine and 3-5-di-brom-tyrosine are essentially negative.

Conversely the administration of 3-5-di-iodo-tyrosine to thyroidless and pituitaryless larvæ brought about very striking results, and in so far as metamorphosis is concerned, simulated the action of thyroid extract, although the effect of the latter is somewhat more rapid. Within a few days after feeding iodo-tyrosine the animals appear thin and emaciated; the limbs grow rapidly and the other changes incident to metamorphosis appear. Twenty days after the date of first feeding the entire culture of thyroidless animals had completed metamorphosis except for the loss of the tail. The pituitaryless animals developed fore and hind legs, frog mouths but invariably died before tail resorbtion was complete. In two pituitaryless animals the right fore legs broke through the skin eight days after the animals were placed upon the iodo-tyrosine diet. These two

individuals were exceptional in this respect, the average time being about twenty days.

These results are of significance for several reasons: 1. The experiment is clear cut and admits of but one interpretation, *i. e.*, that it is the iodine within the tyrosine molecule that is essential for Anuran metamorphosis, because tyrosine without the two atoms of iodine is incapable of inducing metamorphosis.[1]

2. Thyroidless and pituitaryless frog larvæ do not metamorphose unless fed thyroid substance or very large quantities of elemental iodine. Such larvæ have no thyroid mechanism for the manufacture of the thyroid hormone, yet apparently are able to utilize inorganic iodine when administered in very large quantities along with normal food. The substitution of the two iodine atoms for two hydrogens of the tyrosine molecule transforms the tyrosine into a highly active metamorphosis-inducing agent far superior to any quantity of elemental iodine in its rate of action and second only to the thyroid hormone itself. It seems clear that in tadpoles metamorphosis depends upon an organic iodine complex of some sort, and that the iodine to be effective does not need to undergo transformation within the thyroid gland. It is evident that when thyroidless larvæ metamorphose when fed quantities of inorganic iodine, the latter to be active must enter into organic combination with either the body proteins of the larvæ or the algæ fed with it.

3. Iodized proteins and amino-acids have been employed by several investigators in the treatment of various disorders of the thyroid in cases of human hypothyroidism, but the writer has never heard that such products can completely take the place of the thyroid hormone. Yet in thyroidless Anuran tadpoles iodo-tyrosine to all intents and purposes is as good as the thyroid secretion in transforming the individual, *i. e.*, it completely takes the place of the gland secretion in so far as metamorphosis is concerned.

Metamorphosis in Anurans is dependent upon a peculiar property of the iodine atom when organically combined in a certain way and it seems that mammals and Anurans are not to be compared in regard to their reactions to iodine. Thyroidless tadpoles promptly metamorphose when fed iodized amino-acids or large quantities of elemental iodine, but thyroidless mammals and individuals with atrophied and very degenerate glands can not utilize elemental iodine at all, and the same is probably true of iodized amino acids. Voegthlin and Strouse ('09, *Journal Pharm. and Exp. Therap.*) observed that iodized amino-acid fails to replace the thyroid function in pathological cases of hypothyroidism, *i. e.*, myxedematous and cretinous mammals, nor was the nitrogen metabolism or blood pressure of dogs influenced by administration of this substance. Furthermore, Miura ('22, *Jour. Lab. and Clinical Med.*, Vol. 7) has made the interesting observation that di-iodotyrosine gives no protection to mice against lethal doses of acetonitrile, whereas thyroid tissue protects these animals against the poison.

It is clear that in so far as the metamorphosis of thyroidless and pituitaryless tadpoles is concerned iodo-tyrosine is practically as good as the thyroid hormone but this same substance in mammals can not be substituted for the glandular tissue itself.

4. The suggestion of some recent writers that bromine if it could be substituted for the iodine of the thyroid might prove just as active physiologically, is not borne out by these experiments. Bromine has no influence upon Anuran transformation and can not be substituted for iodine.

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[1] Large axolotls seven inches long were *thyroidectomized* and kept five months following the operation and then twice injected with iodotyrosine. Metamorphosis resulted within seventeen days following the first injection. Similar animals injected with tyrosine and dibromtyrosine did not transform. Partially thyroidectomized axolotls (two thirds of the gland excised) were kept five months and then twice injected with iodo-serum globulin. Metamorphosis occurred within sixteen days. Uhlenhuth's claim that Urodele larvæ differ from Anurans in regard to metamorphosis and iodine is not sustained.